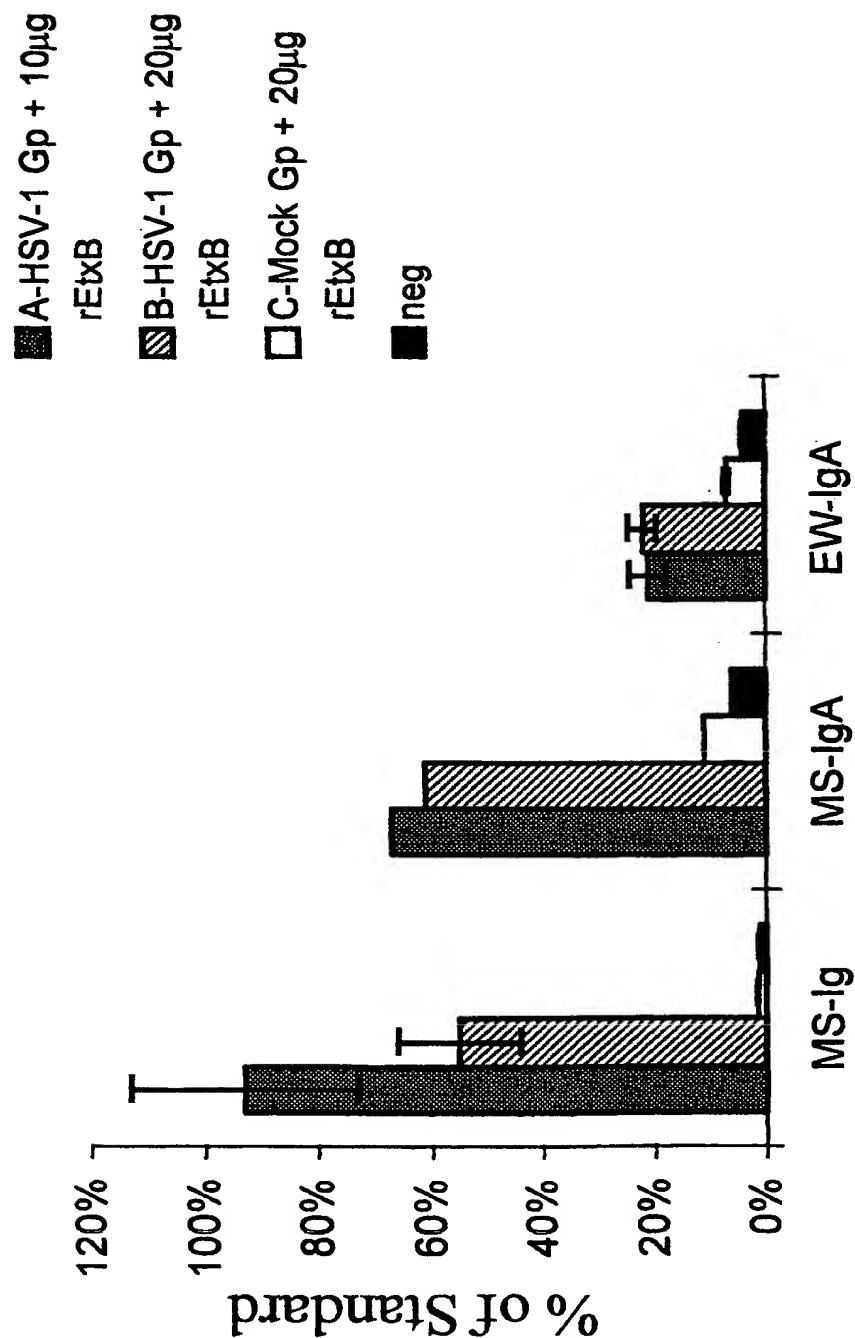


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FIGURE 1

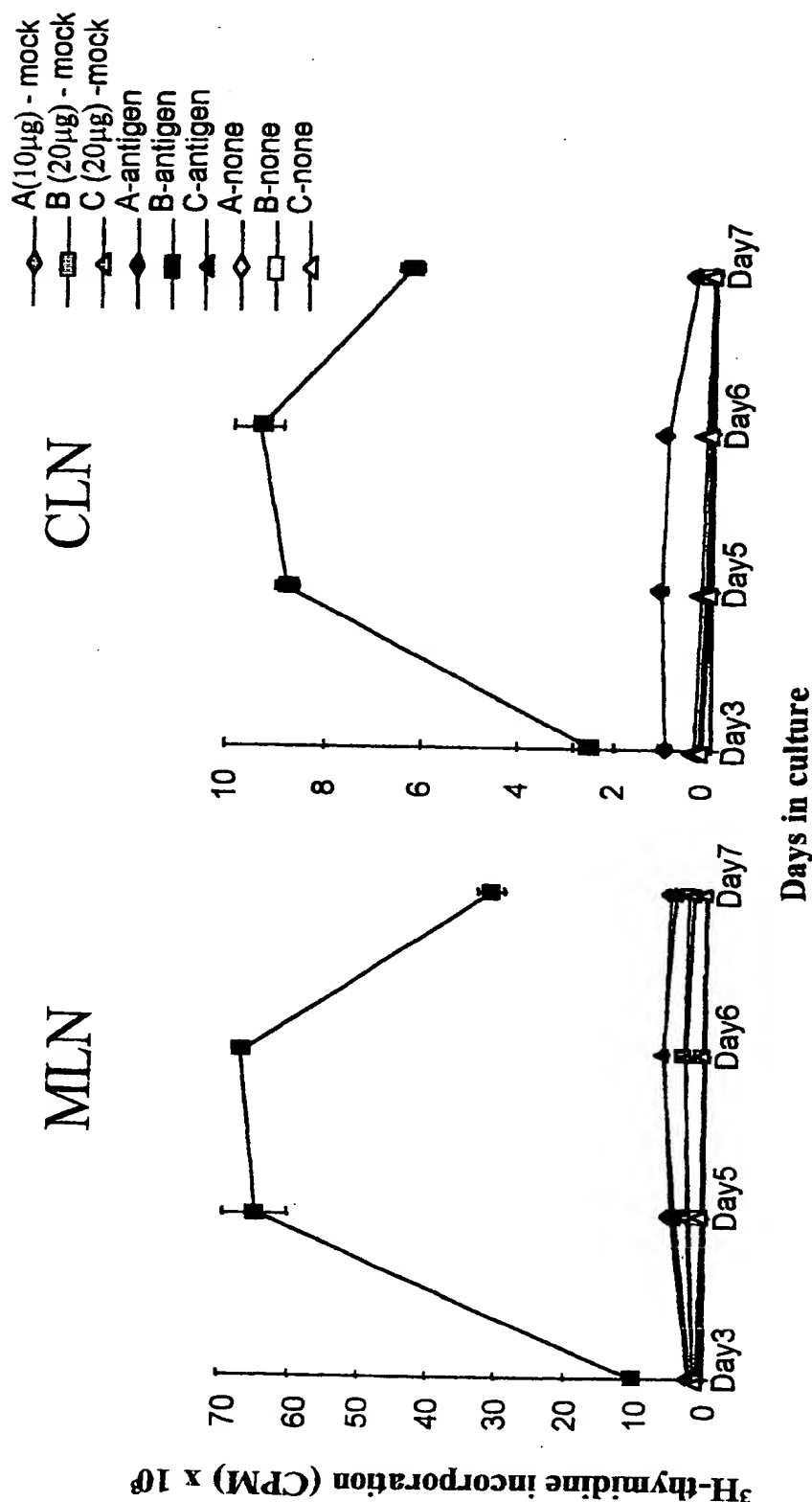
Level of Ig or IgA in MS or IgA in EW compared with control mice following immunisation with HSV-1 or mock Gp preparations with different amounts of rEtxB



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FIGURE 2

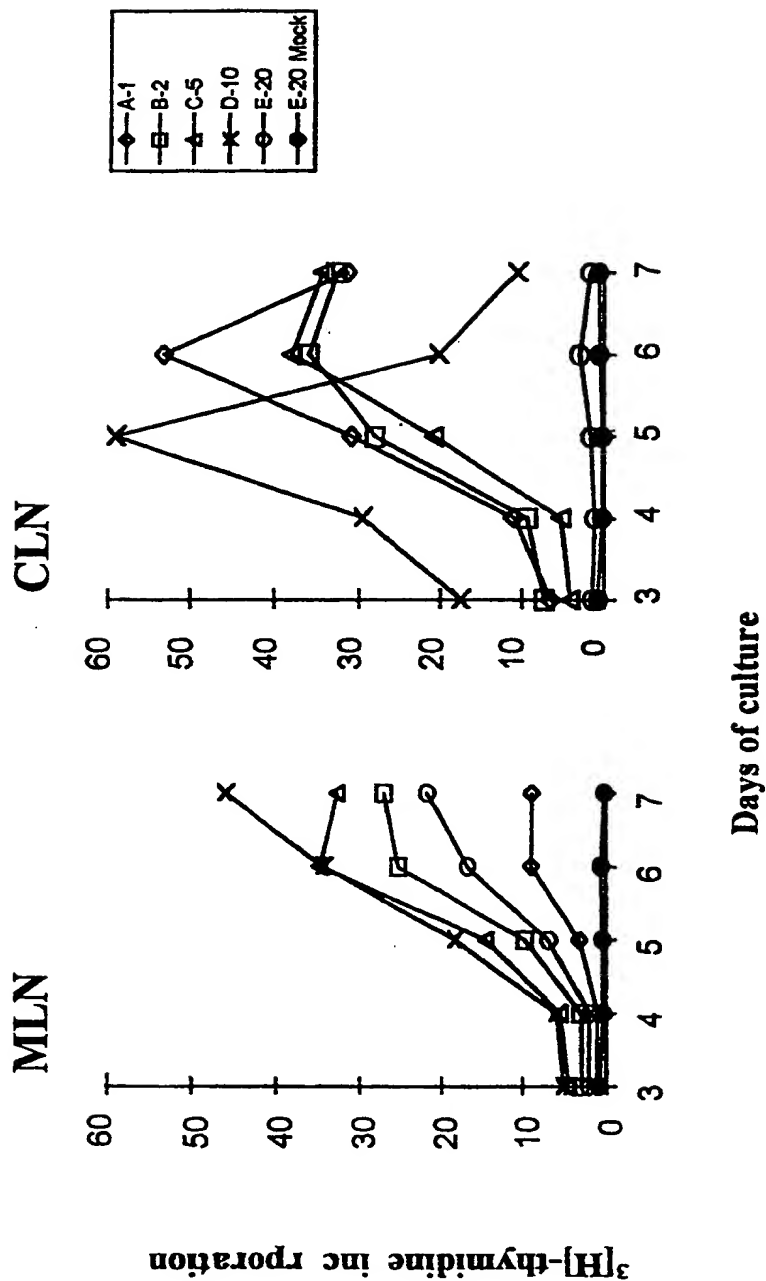
T cell proliferation of MLN or CLN lymphocytes from mice given HSV-1 glycoproteins (gp) with 10 $\mu$ g (A), 20 $\mu$ g (B) rEtxB or mock Gp with 20 $\mu$ g rEtxB (C) by the i.n. route cultured *in vitro* with HSV-1, mock or no antigen



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FIGURE 3

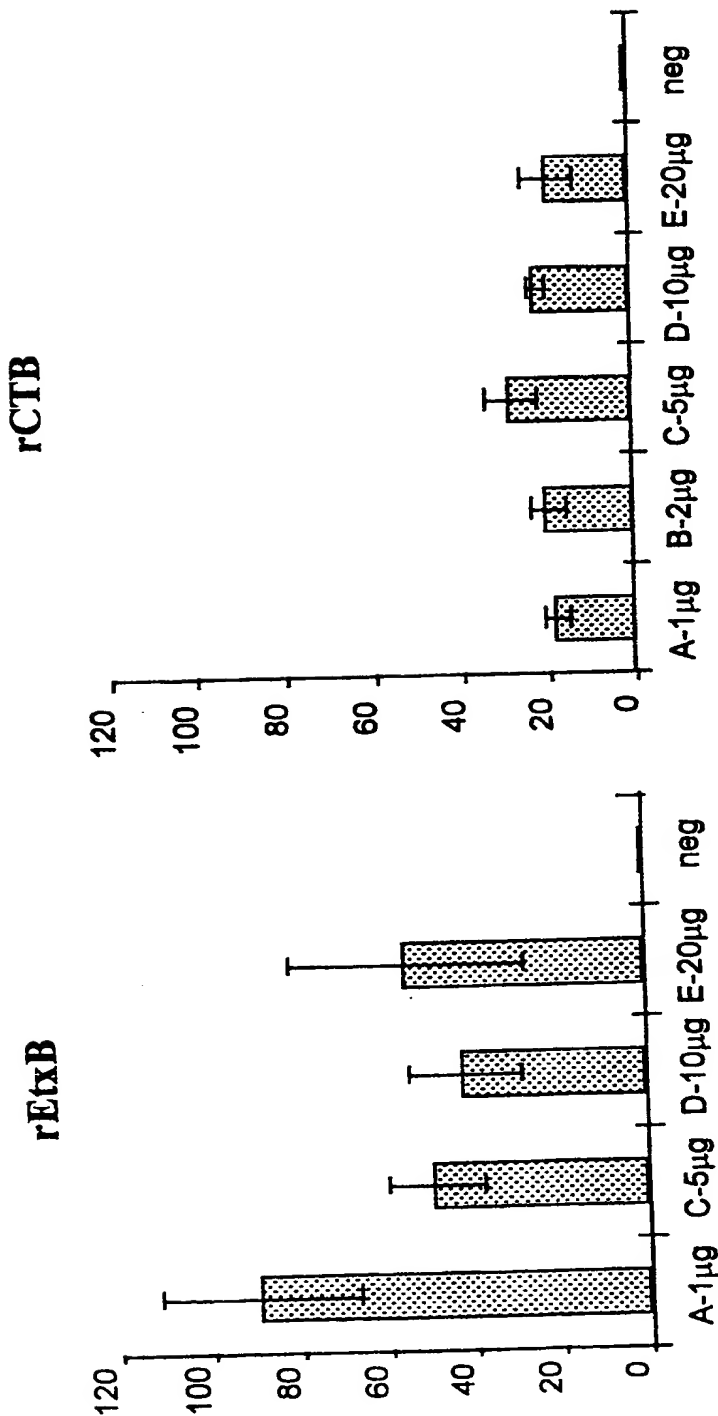
Tcell proliferation of cells from MLN and CLN of mice immunised i.n. with HSV-1 Gp in the presence of 1-20µg EtxB as adjuvant



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FIGURE 4

Anti-HSV-1 serum Ig in mice following administration of HSV-1 glycoproteins three times at 10 day intervals with variable amounts of rEtxB or rCTB as adjuvant



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**Figure 5a. Incidence of virus shedding from the eye following corneal scarification of mice with HSV-1 (SC16)**

Day post infection	10µg rEtxB + HSV-1 gp (%) <sup>1</sup>	20µg rEtxB + HSV-1 gp (%)	20µg rEtxB + mock gp <sup>2</sup> (%)
1	0	30	60
2	60	80	95
3	60	80	95
6	10	0	70
7	10	0	70
8	0	0	10
9	0	0	0

<sup>1</sup> Percentage of animals from which wash fluid from the eye secretions revealed the presence of live viral particles in a plaque assay.

<sup>2</sup> Mock infected animals were given an inoculum of glycoproteins prepared from uninfected tissue culture cells.

**Figure 5b. Clinical disease following corneal scarification of mice with HSV-1 (SC16)**

	Corneal ulcers <sup>2</sup>	Oedema	Lid disease	Zosteriform infection	Encephalitis	Latency <sup>1</sup>		
						TG1	TG2	TG3
10µg rEtxB + HSV-1 gp	80%	0%	0%	0%	0%	22%	11%	0%
20µg rEtxB + HSV-1 gp	70%	0%	0%	0%	0%	80%	10%	0%
20µg rEtxB + mock gp	80%	45%	55%	40%	40%	83%	30%	16%

<sup>1</sup> Latency was determined by extraction of the trigeminal ganglion (TG) from surviving mice 2 months after infection and coculturing with Vero cells. Figures given are for each of the lobes of the TG (TG1, TG2 and TG3).

<sup>2</sup> Figures are percentage of animals showing signs of the described symptoms at any point during acute infection. Each mouse was examined on a daily basis during the first 11 days of infection.

N=15 per group

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FIG. 6

**Ig Isotype distribution in MS from mice following infection (pos) or immunisation with HSV-1 Gp in the presence of EtxB or CTB as adjuvant**

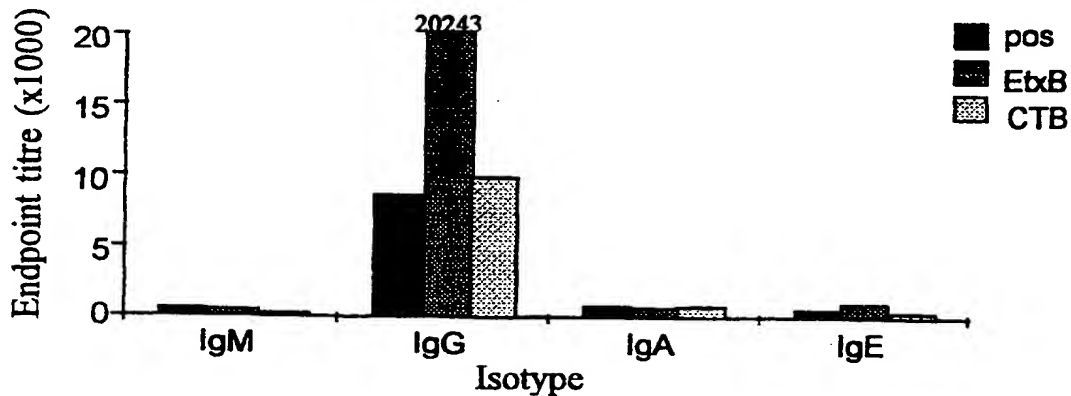
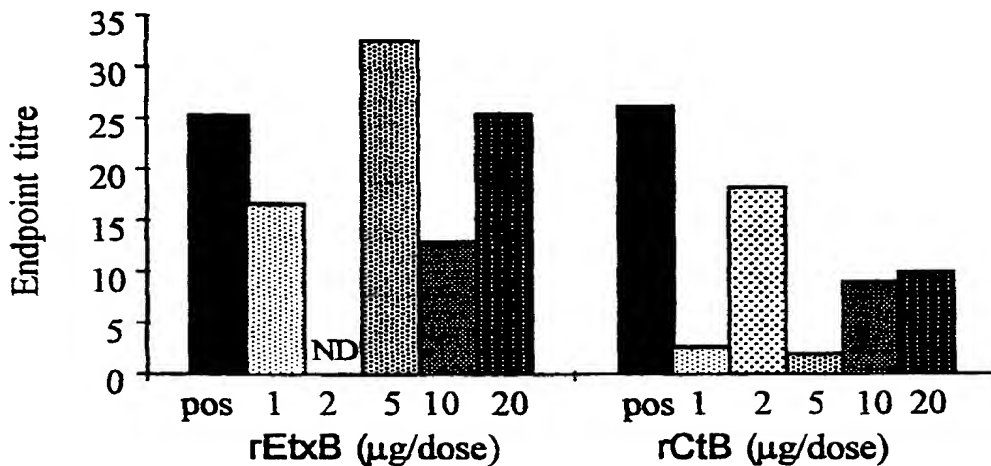
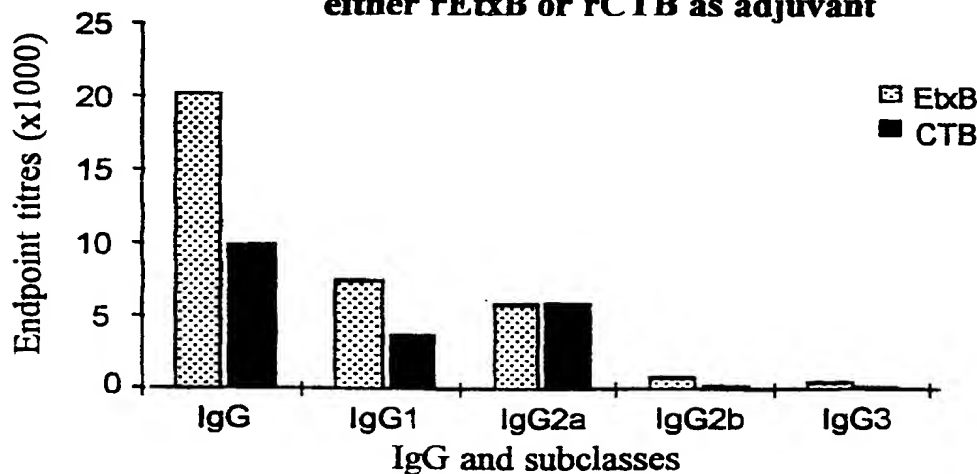


FIG. 7

**Adjuvant effect of different amounts of rEtxB or rCtB on the level of HSV-1 specific IgA in eye washings following administration with HSV-1 glycoproteins**

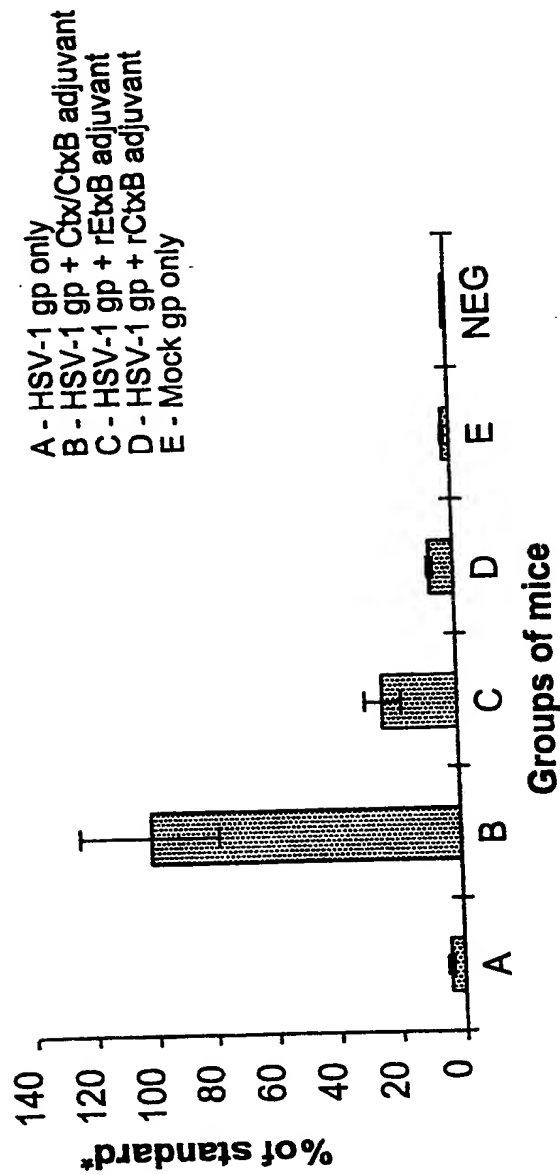


**FIG 8. Distribution of subclasses following administration of HSV-1 Gp i.n. with either rEtxB or rCTB as adjuvant**



**FIGURE 9**

**Serum immunoglobulin response following immunisation of mice with HSV-1 or mock glycoproteins (gp) alone or in the presence of adjuvant**



\* antibody levels were measured by ELISA and are expressed as a percentage of the levels stimulated following ocular infection induced by scarification with  $10^5$  pfu HSV-1 strain SC16.

Ctx/CtxB = 0.5  $\mu$ g Ctx + 10  $\mu$ g CtxB

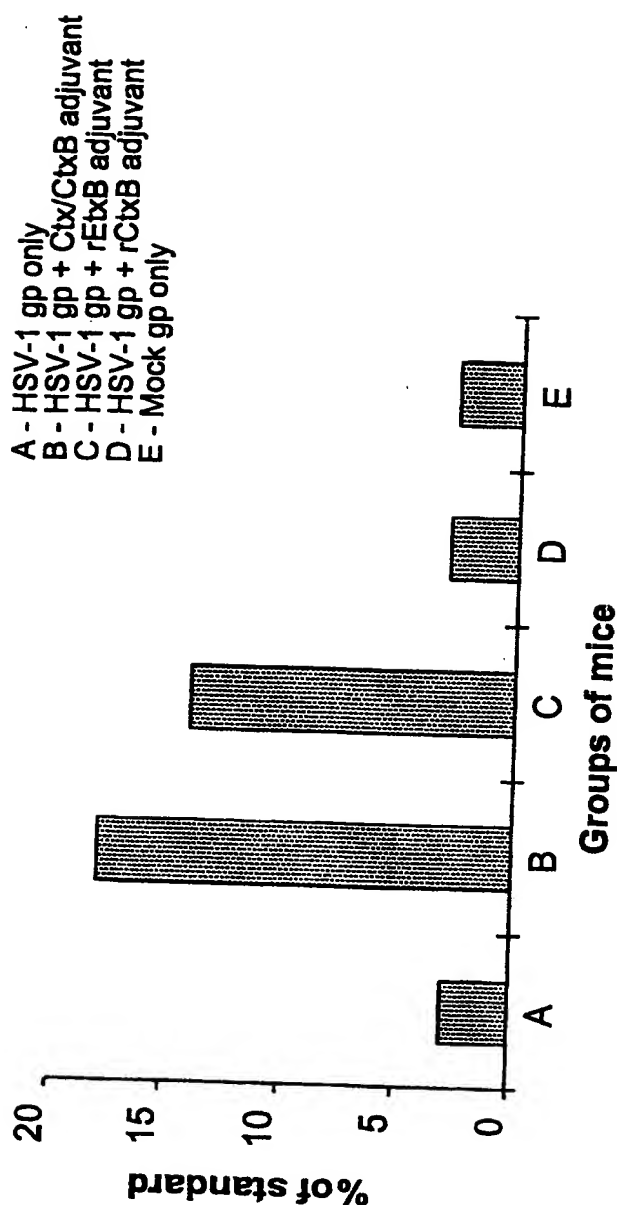
rETvB = 10 $\mu$ g recombinant EtxB

gp = 10 $\mu$ g HSV-1 or mock glycoproteins as indicated.

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FIGURE 10

# Mucosal IgA in eye washings following intranasal immunisation of mice with HSV-1 or mock glycoproteins (gp) alone or in the presence of adjuvant



\* antibody levels were measured by ELISA and are expressed as a percentage of the levels stimulated following ocular infection induced by scarification with  $10^5$  pfu HSV-1 strain SC16.

Ctx/CtxB =  $0.5\mu\text{g}$  Ctx +  $10\mu\text{g}$  CtxB

rEtxB =  $10\mu\text{g}$  recombinant EtxB

gp =  $10\mu\text{g}$  HSV-1 or mock glycoproteins as indicated.

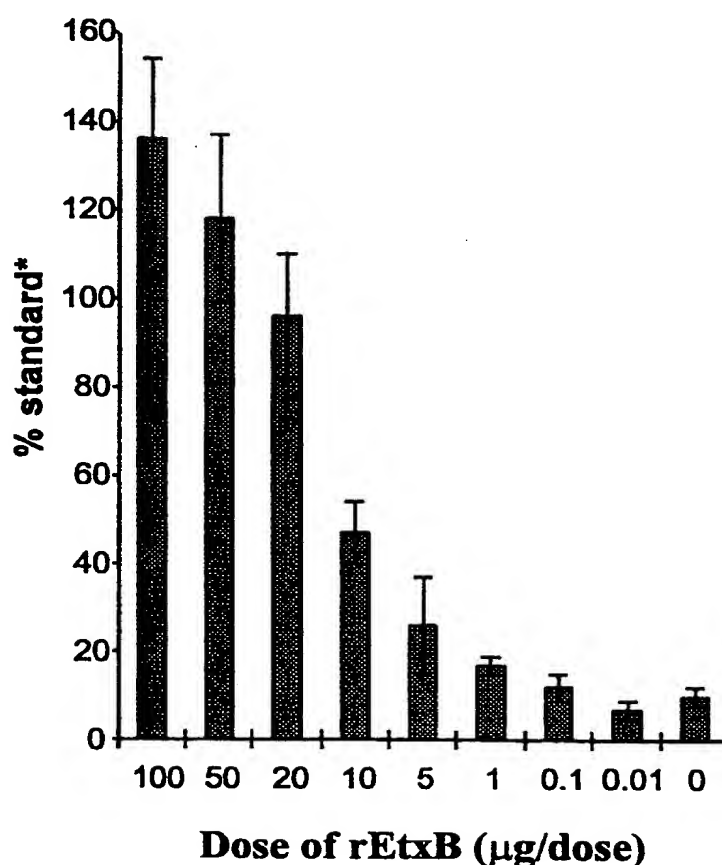




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FIGURE 12

**Level of HSV-1-specific immunoglobulin in sera from mice immunised with HSV-1 glycoproteins in the presence of different doses of rEtxB as adjuvant**

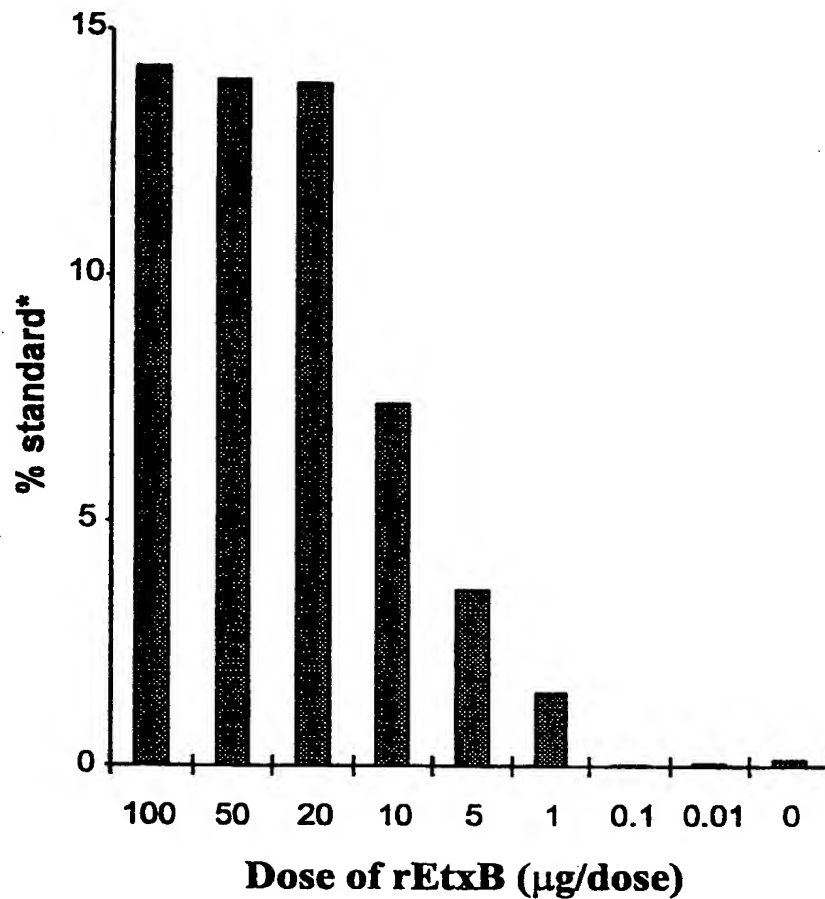


\* antibody levels were measured by ELISA and are expressed as a percentage of the levels stimulated following ocular infection induced by scarification with  $10^5$  pfu HSV-1 strain SC16.

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FIGURE 13

**Level of IgA in eye washings of mice immunised with HSV-1 glycoproteins in the presence of varying concentrations of rEtxB**

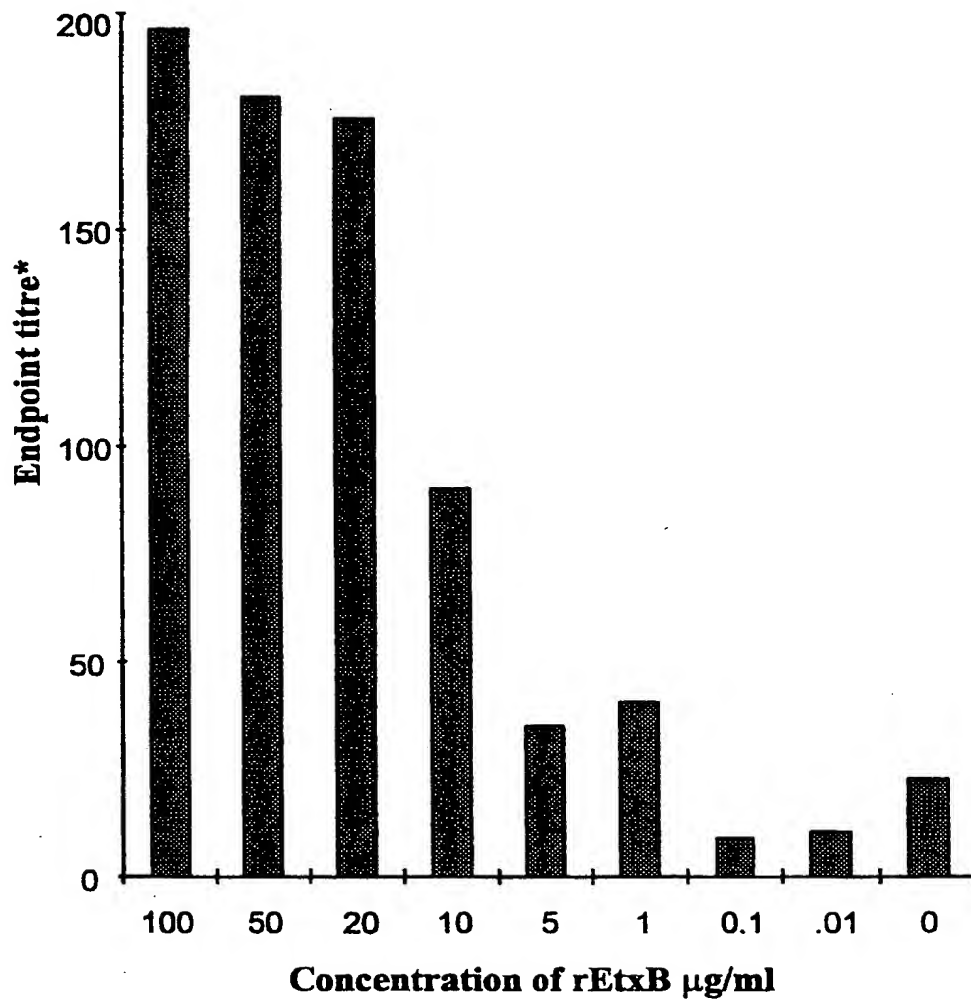


\* antibody levels were measured by ELISA and are expressed as a percentage of the levels stimulated following ocular infection induced by scarification with  $10^5$  pfu HSV-1 strain SC16.

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FIGURE 14

**Level of IgA in vaginal washings of mice immunised with HSV-1 glycoproteins in the presence of varying concentrations of rEtxB**

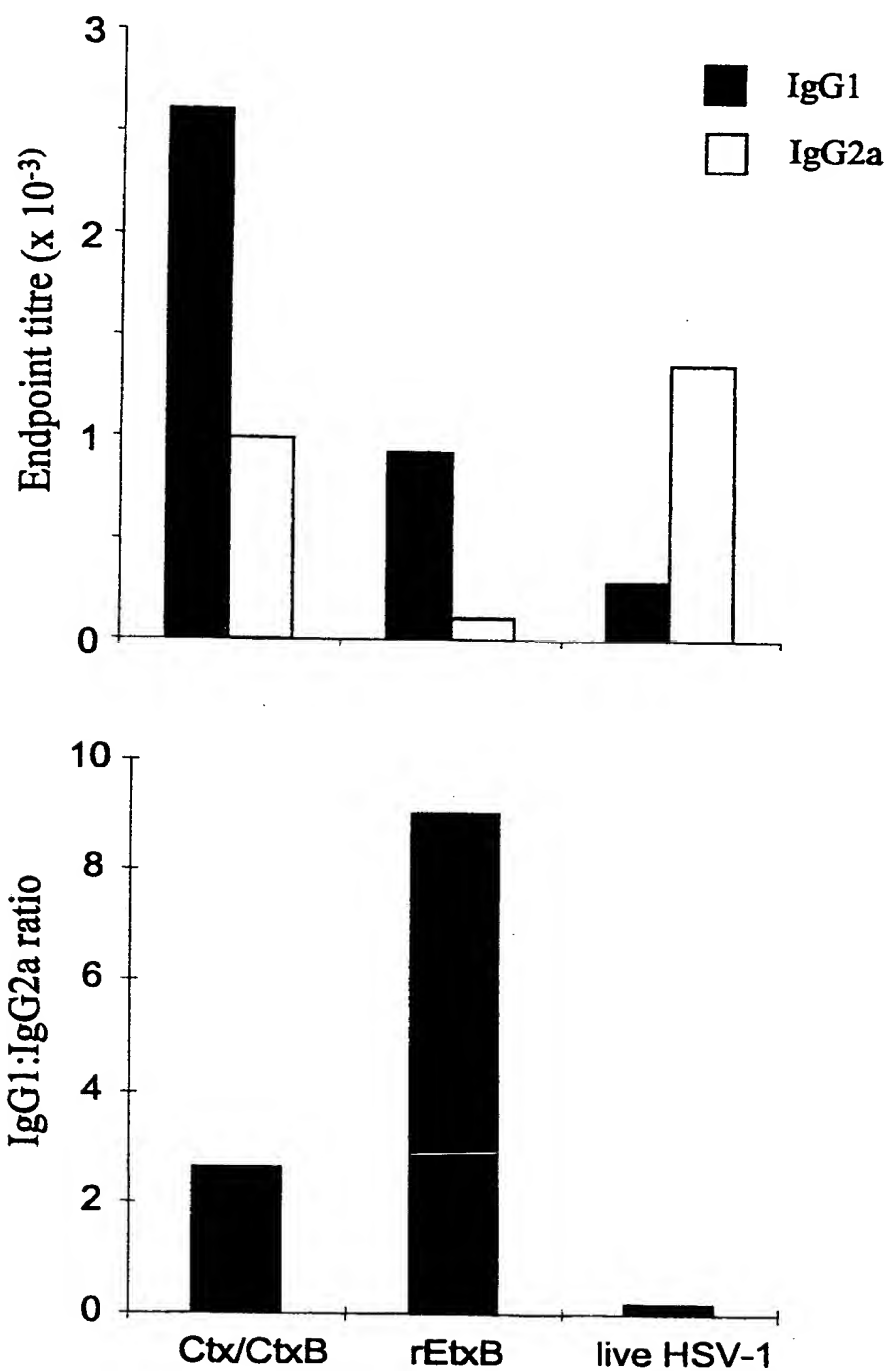


\* antibody levels were measured by ELISA and are expressed as endpoint titres calculated using linear regression analysis.

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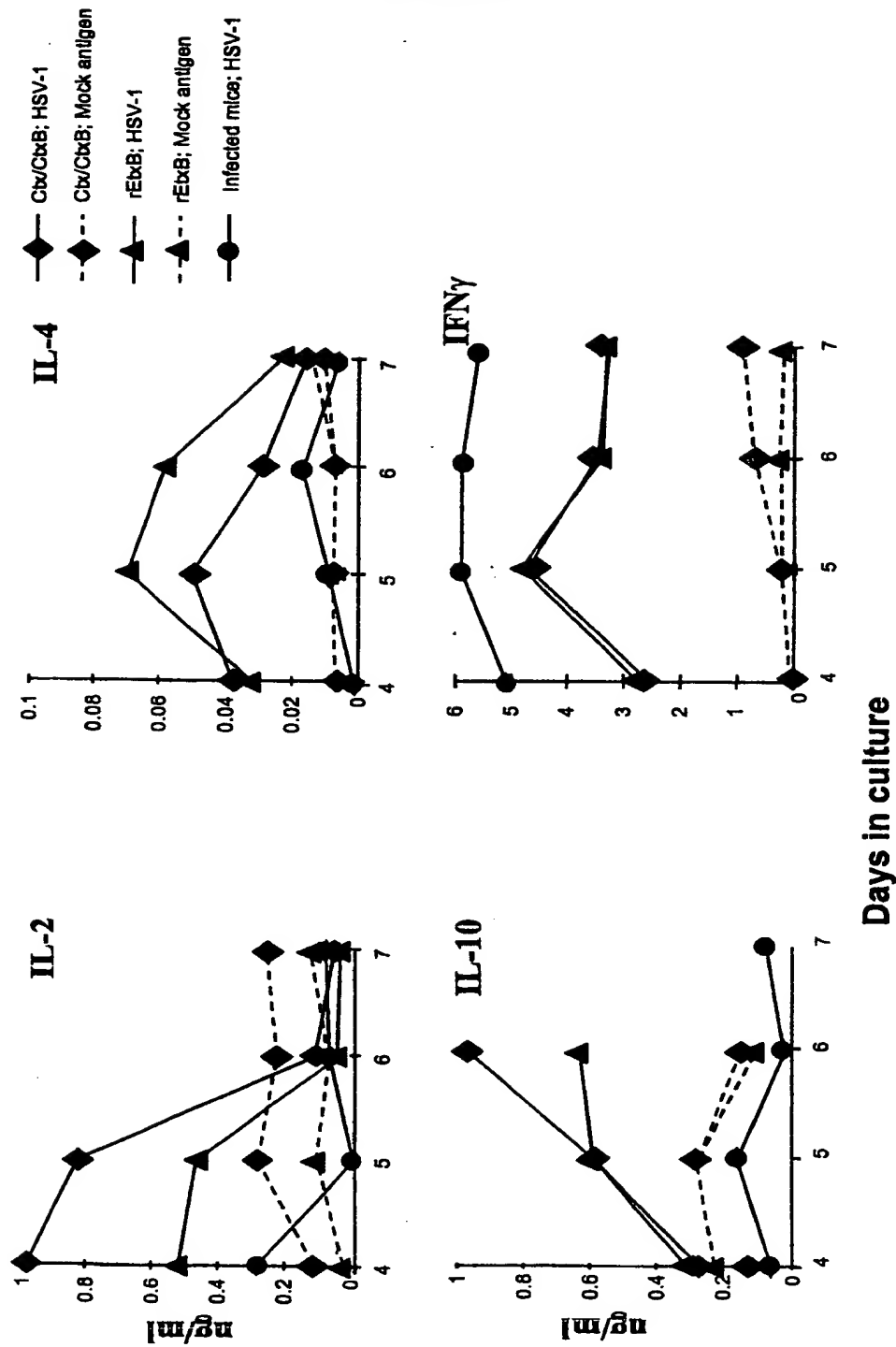
FIGURE 15

IgG subclass distribution of the serum antibody response to HSV-1 following intranasal immunisation with Ctx/CtxB or rETxB or ocular infection with HSV-1



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FIGURE 16



Cytokine production from cultures of lymph node cells taken from mice which were either infected with HSV-1 by ocular scarification, or were immunised by intranasal administration of HSV-1 glycoproteins with either Ctx/CtxB or rEtxB as adjuvant

Cytokines were measured using cELISA and quantities calculated against standard curves prepared using recombinant cytokines. Values are expressed from cultures from mice immunised intranasally with 10µg HSV-1 glycoproteins with either Ctx/CtxB or rEtxB as adjuvant, and cultured with whole killed HSV-1 (HSV-1) or identically treated mock virus preparation (Mock antigen).

FIGURE 17

Level of protection against ocular HSV-1 infection in mice immunised intranasally with a mixture of HSV-1 or mock glycoproteins in the presence of rEtxB as adjuvant

Immunisation	Corneal Ulcers	Opacity/ Oedema	Lid Disease	Zosteriform Infection	Encephal- itis	Latency		
						TGI	TGH	TGHI
10µg HSV-1 gp + 10µg rEtxB per dose	69%	10%	0%	3%	0%	22%	11%	0%
10µg mock gp + 10µg rEtxB per dose	80%	68%	74%	72%	50%	83%	30%	16%

<sup>1</sup>n=29  
<sup>2</sup>n=30